The Apoplast of higher plants: Compartment of Storage, Transport and Reactions

The Apoplast of Higher Plants: Compartment of Storage, Transport and Reactions

The significance of the apoplast for the mineral nutrition of higher plants

edited by

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IN MEMORY OF BURKHARD SATTELMACHER

Burkhard Sattelmacher was an internationally highly estimated scientist in the area of plant mineral nutrition. He contributed substantially to the scientific excellence of Plant Nutrition especially through his engagement within the German Research foundation particularly through the initiation and contribution to coordinated research programmes and as a member of the International Council for Plant Nutrition. He was a stimulating teacher, mentor, and colleague. He found research in plant nutrition fascinating, and was able to transmit that fascination to those around him. He died in November 2005 at the age of 58 after many months of courageous fighting against his disease.

Born in Kiel he studied Botany at the Technical University of Berlin. He got his PhD in Plant Nutrition at the same University under the guidance of Horst Marschner. Deeply concerned about poverty alleviation through plant-production research he continued his work on the physiology of potato for 4 years as a post doc at the International Potato Center (CIP), Lima, Peru. This and follow-up research in Hohenheim represented the basis for his habilitation at the University of Hohenheim in 1986. In 1985 he accepted the call as professor for Plant Nutrition in Kiel. Since 1992 he was head and chairholder of Plant Nutrition at the Institute of Plant Nutrition and Soil Science, Faculty of Agricultural and Nutritional Sciences, University of Kiel.

In the centre of the scientific interest of Burkhard Sattelmacher was the physiology of crops. He was convinced that its basic understanding is a prerequisite for solving practical problems related to crop management. In the early nineties Burkhard Sattelmacher developed a research area on nutrient fluxes in agricultural land-use systems comparing conventional and "biological" plant-production systems. Over 9 years he participated in a German Research Foundation (DFG)-funded Special Research Project with research projects on root turn-over, N uptake particularly from manure, ammonia and dinitrogen-oxide emission in a winter rape-seed winter-barley rotation. He extended his interest to the nutrient budgets of natural ecosystems in the ecosystem research programme Bornhöveder Seenkette.

Among the research projects he initiated during the last years were particularly two to which he devoted his full force until the last days of his life: the DFG Special Research Programme "The apoplast of higher plants: compartment of storage, transport, and reactions" and the DFG Research Group "Matter fluxes in grasslands of Inner Mongolia as influenced by stocking rate".

Burkhard Sattelmacher always maintained an interest in new developments in agronomy, botany, and soil science. He especially enjoyed discussing ideas with colleagues and students. He was highly estimated as a referee for scientific journals, as well as for funding agencies not only because of his wide knowledge and experience, but because his interest was in the progress of science, without personal bias.

We have lost in Burkhard Sattelmacher an extraordinary person, teacher, scientist, and colleague. We will miss his stimulating contributions to scientific progress. The co-editor and the authors dedicate this book summarising the main achievement of the special research programme "Apoplast" which he initiated and of which he was the speaker. Unfortunately, he did not have the pleasure to finish this book himself.

Walter. Horst

CONTENTS

PREFACE

It was the botanist Ernst Münch, who separated the plant into two principal compartments, the "dead" apoplast and the living symplast. While Münch thought, that water and solute transport were the sole functions of this new plant compartment, we know today that apoplastic functions are much more diverse. It has been suggested to consider "the apoplast as the internal physiological environment of plant bodies", that essentially maintains homeostasis. The response to phytohormones such as auxins or pathogen attack may illustrate that in many cases environmental stimuli are not received directly by the cell, but perceived via changes within the plant's internal environment.

It is not before the last 20 years, that the cell wall attracted the interest of a broader group of plant scientists. It soon became evident, that the term cell wall may be misleading, since it is not appropriate for a highly complex and flexible matrix consisting of cellulose, hemicellulose, pectins and proteins interacting with metabolites. By now we know that the chemical and physical properties of cell walls are not static but depend on a number of parameters including ontogeny and environmental parameters such as temperature, light, nutrient supply, and biotic and abiotic stresses. This is why it was suggested to replace the term "cell wall" by the more precise term "extracellular matrix". The more we learned about the extracellular matrix, the more it became apparent that only few processes during growth and development of a plant do not involve cell walls.

From the viewpoint of plant mineral nutrition, the apoplast is of interest in many respects: nutrients do not simply pass through the apoplast before being taken up into the symplast, but they may also be adsorbed to cell-wall components, complexed, and oxidized/reduced which may be of significance for nutrient acquisition, nutrient function and tolerance of deficiency and toxicity stresses. Also the regulation of long-distance ion transport in the apoplast, the xylem, is not understood. However, this process is of great significance for the understanding of deficiency and toxicity symptoms.

The book summarizes the experimental work conducted during a trans-disciplinary research programme funded for 6 years by the German Research Foundation (DFG) within the Priority Research Project SPP 717. This financial support is highly acknowledged.

In their contributions, the authors from different disciplines not only report original research but also review the state of knowledge in their particular research fields: nutrient acquisition, short and long distance (xylem) transport, tolerance of nutrient deficiencies and mineral toxicities, and the role of micro-organisms colonizing the apoplast. Introductory

remarks are written to each of the sections by internationally highly recognized scientists in their research areas.

We hope that this book will contribute to stimulate further research leading to a better understanding of the role of the apoplast in plant mineral nutrition.

Burkhard Sattelmacher[†] Walter J. Horst

Foreword

The Plant–Leaf Apoplast

THE PLANT–LEAF APOPLAST

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Abstract. The general properties of the extracellular matrix (the cell wall) in roots and leaves are compared and it is suggested that there may be several components of the apoplast in either tissue system where barriers such as the endodermis and bundle sheath are present. There can be very large amounts of xylem fluid delivered to leaves, all of which must pass through the apoplast at some point. The apoplast is a relatively small volume and processes such as ion uptake into the cells, phloem export and sequestration in the matrix are important in preventing a rapid build-up of extracellular solutes. The techniques developed to study such matters are briefly described. The proposition that the apoplast can serve as a store for nutrients is examined. Invasion of the apoplast by endophytic organisms and by environmental pollutants and toxic metals provokes an intriguing cascade of responses, some of which increase the resistance of the plant to biotic and abiotic stress.

1. INTRODUCTION

The extra-cellular matrix of the walls around most living cells is porous, pores being waterfilled in all but very exceptional circumstances. Between the cells of the root cortex leaf mesophyll there are much larger spaces. In leaves, carbon dioxide, oxygen and water vapour diffuse through these spaces, the former gases dissolving in the water film around the cells while the latter evaporates from the surfaces. It is possible to give a misleading emphasis to the differences between the root and leaf apoplasts due to these prominent spaces. In actuality, the water layers associated with cell surfaces and the porosity, chemical composition and electrostatic properties of the walls are of a similar order in both tissue systems. The cardinal difference is that water in the apoplast of the root cortex is usually continuous with the bulk water in the external medium while in leaves it is not. Continuity of water allows diffusion of solutes into and out from a root, even when the root has a barrier resistance created by an exodermis (Hose et al., 2001).

B. Sattelmacher† and W.J. Horst (eds.), The Apoplast of Higher Plants: Compartment of Storage, Transport and Reactions, 3–12.

Thus, materials that accumulate, for one reason or another, in the root apoplast can be unloaded directly into the external medium. This cannot occur in leaves unless a substance is volatile. Discharging other sorts of materials from leaves can occur only if they can reach, and be loaded into the phloem. For some very important inorganic ions, e.g. Ca^{2+} and H^{+}, this does not occur to any significant extent.

In roots there are at least two apoplast components that are separated by the endodermis. Thus there is a cortical apoplast and a stelar apoplast; the xylem contents may be regarded as part of the latter system. There is an analogous structure, the bundle sheath, separating the extracellular space within the leaf veins and the apoplast of the mesophyll. This structure is present in many species and is by no means restricted to the C4 species in which it has a special significance is separating stages of carbon fixation in photosynthesis. In leaves, therefore, we may find that there are two quite separate apoplast compartments. This has relevance in the discussion of methodology of apoplast compositional analysis.

The above, fairly obvious, points bear on some of the principal difficulties that readers will encounter in the sections that follow in this book. In pre-empting that discussion, we might ask ourselves how the conditions can be regulated in such a highly ramified, but small space can be measured reliably, how those conditions can be regulated and how the apoplast might serve as a storage compartment?

2. SOME PHYSICAL FACTS

Using several averaging, extractive techniques (see below) estimates of the volume of the apoplast in leaves are in the range of 4–11% of the leaf fresh weight. Thus, in a leaf weighing 1 g FW the expected apoplast volume might be 40–110 μl. Much of the water in this volume is present in pores present in the interstices of interwoven cellulose micro fibrils of the wall, or more properly, the extracellular matrix. These pores have been estimated to be in the order of 3–5 nm diameter (Gogarten, 1988; Sattelmacher, 2001). The porosity of the wall can be imagined in both the vertical plane, in which case the pores will usually be no more than 1–3 μm in length or in the horizontal plane in which case they can be enormously longer, and highly tortuous (Clarkson, 1991). Diffusion of solutes in this horizontal plane will be greatly hindered because of chemical interactions with the pectins of the middle lamella and by the great length of the pores. These considerations suggest that it may be possible for considerable gradients of solute concentration to build up at tissue sites where there may be rapid efflux of materials from the cells.

Close to the surface of the plasma membrane there are water layers whose exact physical state is hard to measure. Water molecules closest to the plasma membrane will be bound to the surface and have a significantly greater viscosity than bulk water, resembling liquid ice. Water molecules can be exchanged readily between this layer and the "free" water beyond it. As long as the rate constant for this exchange is faster than that for water movement across the plasma membrane, the bound water layer will be kinetically invisible. For solutes, however, diffusion across the bound water layer(s) involves movement through a layer of greater viscosity than in "free" water and solute transfer across the layer will be slowed down.

Rates of xylem delivery of water and solutes to leaves during the day can be large relative to the volume of the apoplast. Consider that it would not be uncommon for the mass of water lost by evapo-transpiration during the day to be five times greater than the fresh weight of a leaf. At some point, all of this water must move through the leaf apoplast. In a leaf with FW 1g, the apoplast volume might be as little 40 µl. In an hour during the photoperiod, as much as 500 µl may pass through this compartment. This is an equivalent to a complete volume change every 4.8 min. Since the xylem sap may contain major solutes, such as K^+, Ca^{2+} and NO_3^- in millimolar concentrations, they must either enter the cells quickly or be translocated out of the leaf in the phloem to prevent a massive build-up of electrolytes in the apoplast. In the special case of leaves receiving a heavy input of salt because they are in a saline medium, this may occur so that the water potential between the apoplast and the cells will be decreased (Flowers et al., 1991).

3. MEASUREMENTS OF APOPLAST COMPOSITION

The foregoing description of some of the physical features of the apoplast indicates two things. First that a method that produces a general composition of the fluid that can be extracted from the apoplast may obscure important gradients of composition between sites in the leaf where metabolism and transport activity are more or less intense (see Canny, 1990). Second, that there is a risk that physical intervention into such thin, tortuous layers may report the composition where the apoplast has been disrupted by a probe or electrode. In the sections that follow we will see that either of the above approaches can give consistent results that can be useful in interpreting the relationship between the extra- and intra-cellular environment in leaves. For obvious reasons though, they do not give the complete picture and, at worst, may lead us to heresy in which part of the truth is mistaken for the whole truth.

The earliest attempts to analyse apoplast fluids made use of centrifugation or displacement (e.g. Bernstein, 1971). Another approach to

estimate the free space composition of leaves made use of tracer wash-out techniques that were the exact counterparts of those employed in transport kinetics of root segments. Leaves, preloaded with radioactive tracers, were cut into fine strips and suspended in a non-radioactive bathing medium that was sampled and changed at intervals (Pitman et al., 1974). This allowed the kinetics of the tracer exchange to be measured and the ionic content of various pools, or phases to be estimated. These techniques showed that the general exchange properties of leaf cells, in these conditions, were quite similar to those of roots.

In the sections that follow, the reader will find several examples of methods for estimating the composition of the solution in the apoplast. Centrifugation/displacement techniques are evaluated in the paper by Lohaus (this volume, pp. 323–336) and in exemplary studies published earlier (Dannel et al., 1995; Lohaus et al., 2001). In addition there is direct intervention of micro-electrode probes to measure ion activities (Felle and Hanstein, this volume, pp. 295–306), and ion-specific fluorescent probes that can be targeted, in transgenic plants, to the apoplast (Plieth et al., this volume, pp. 373–392).

Evidently, when a detached leaf is centrifuged, with its petiole parallel to the centrifugal force, a number of fractions of different composition may be gathered (e.g. Dannel et al., 1995). The first will comprise the contents of the xylem vessels, followed by the solution obtained from the apoplast around the leaf veins (or within the bundle sheath, where this is present). Later fractions will contain the solution migrating from the walls. In the paper of Lohaus (this volume, pp. 323–336) this fractionation is described and certain markers enzymes, that indicate actual damage to the cells, are assayed (Dannel et al., 1995). Clearly, evidence of damage invalidates the whole approach.

Ion selective micro electrodes, with several barrels that can measure two or three ion activities simultaneously, can be introduced into leaves via the open stomata (Felle et al., 2000). This allows the electrode tip to make contact with mesophyll cells with the minimum of cellular disruption so that is less risk that ion leakage from damaged cells can contaminate the apoplast fluids. In the paper by Felle and Hanstein (this volume, pp. 295–306), it is shown that reproducible data can be readily obtained from a number of species. The electrodes can be left in position while conditions affecting the leaf are changed. Thus continuous recording of changes over several hours can be obtained. The results are, however, from a very small, specific site in a widely dispersed compartment. Unless a great deal of sampling is done, it is hard to see how significant gradients of composition in the apoplast can be detected. The physiological significance of this approach would be greatly increased if leaves if intact plants could be attached to measuring cuvettes.

Plieth et al. (this volume, pp. 373–392) developed the use of ion-specific fluorescent indicators in a very promising direction. Various indicator dyes have been available for some time and assessments have been made of leaf apoplast pH (Hoffmann and Kosegarten, 1995), K^+ (Mühling and Sattelmacher, 1997), Ca^{2+} (Mühling et al., 1997) and Na^+ (Mühling and Läuchli, 2002). The technique can reveal micro-variation in the activity of an ion where it can be combined with *in vivo* ratio-imaging techniques. The problem about the exact points of origin of the fluorescent signal can now be tackled by combining a newer generation of fluorescent proteins, e.g. aequorins and green fluorescent protein, with targetting sequences and expressing the genes that encode them in transgenic plants. The potential of this exciting approach is demonstrated by Plieth et al. (this volume, pp. 373–392). The approach should be able to reveal some of the subtlety of the dynamics of transfers between the apoplast and the cell interiors. It depends, however, on the availability of transformable species and, ideally, the optical sectioning of intact leaves. For the time being, these two constraints limit the widespread application of the technique.

4. STORAGE IN THE APOPLAST?

In the sections that follow, the significance of accumulations of silicon (Wiese et al., this volume, pp. 33–48), boron (Wimmer and Goldbach, this volume, pp. 19–32), manganese (Fecht-Christoffers et al., this volume, pp. 307–322), iron (Nikolic and Römheld, this volume, pp. 353–372) and calcium (Felle and Hanstein, this volume, pp. 295–306, Plieth et al., this volume, pp. 373–392) in the apoplast is discussed. At the risk of seeming over-concerned with semantics, it might be said that there is more to a "store" than simply the presence of a given material. In the case of B, for example, the rapidity, with which B-deficiency can occur in growing tissues of plants previously well supplied with B, suggests that the large proportion of plant B harboured in the apoplast is not a very effective store for B, at least, as far as developing cells are concerned. The accumulation of motor vehicles at some bottleneck on the autobahn cannot be properly described as a storage area, whereas an adjacent car park in a rest area might be. The car park, like any kind of store, has a finite capacity and organised entrances and exits. A functional biological store would be a place where material can be deposited or withdrawn in a regulated manner. Central to such a notion is that there should be some control over the process of accumulation. The cell vacuole fits well with these general ideas because fluxes of materials across the tonoplast are regulated by transporters and channels. Similar regulation of transport occurs at the plasma membrane (PM). Perhaps the salient test of the idea that the apoplast can function as a store is that materials accumulating in it can be

mobilized when required and moved either into surrounding cells, or exported for use in another part of the plant. The reader might like to keep this in mind.

It should be remembered that the water-filled spaces in the cell wall comprise a small volume. This places an obvious osmotic constraint on the amount of a solute that can accumulate. Processes such as adsorption, precipitation and polymerization can all reduce the osmotic activity of a material. With a simple salt, such as NaCl, none of the above processes can be expected to play much part in lowering the osmotic strength of the solution in the apoplast, especially when delivery to the leaf is faster than processes such as salt excretion, vacuolar sequestration or export in the phloem. If NaCl accumulation takes place in the apoplast, the cells might respond by the synthesis of compatible solutes, to keep the water potential of the cytosol lower than that of the apoplast, but failing this, one might envisage cells losing turgor. This idea was advanced some time ago by Oertli (1968) and other authors have found evidence to suggesting that cells in the leaf can be damaged by extracellular salt in this way (e.g. Flowers et al., 1991). In the paper by Lohaus (this volume, pp. 323–336) this idea is challenged. This matter provides a suitable project for more refined imaging of Na and probing with Na-selective micro-electrodes.

The xylem stream delivers divalent Mn^{2+} ions to the leaf apoplast. Mn^{2+} is probably the ion species that crosses the plasma membrane of leaf cells via divalent ion channels (Clarkson, 1988). Where the Mn supply is excessive, there appears to be stimulation of peroxidase activity in the apoplast that, in turn oxidizes Mn^{II} to Mn^{III} (Fecht-Christoffers et al., this volume, pp. 307–322). Among other effects, this leads to the accumulation of manganese oxide. In leaves, autoradiography shows that there can be sizeable, discrete deposits of this form of Mn (Horst and Marschner, 1978). There is also a poorly-understood reaction between apoplastic silica and Mn which is probably due to adsorption. This seems to ameliorate the effects of Mn over-supply and toxicity. Both the precipitation and adsorption processes can be viewed as defence mechanisms that may counteract excessive Mn (Rogalla and Römheld, 2002; Wiese et al., this volume, pp. 33–48). Whether or not these accumulations should be regarded as a store is much less certain. Mn in solution will be in a chemically determined equilibrium with Mn adsorbed onto silica and it may be that organic acids can solubilize precipitated Mn oxides, but neither process satisfies the criteria for a useful store.

The situation for Fe is more difficult to interpret. In the paper by Nikolic and Römheld (this volume, pp. 353–372) there is evidence that Fe^{III} iron delivered to the apoplast predominantly as ferric citrate complexes, becomes adsorbed onto polymeric structural components of the mesophyll extracellular matrix. The concentration of free iron in the apoplast fluid appears to be always very low. The authors suggest that the availability of

free Fe^{III} for subsequent reduction (via PM redox system) and transport to the cytoplasm depends on release from the extracellular pool. This equilibrium, affected as it is by factors such as apoplast fluid pH, makes the extracellular iron a buffer; or so it would seem. Nikolic and Römheld (this volume, pp. 353–372), however, found little difference between the quantity of Fe in the extracellular matrix of green and chlorotic leaves of sunflower. This makes it seem that access to the "stored" apoplastic iron may not respond to the biochemical "need" for iron within the cell. Apoplastic iron is not, perhaps, so effective a store as stromal phytoferritin that is rapidly mobilized in greening leaves (Marschner, 1986).

Calcium ions play an essential role in cross-linking elements of the extracellular matrix. They are always present but remain exchangeable with other divalent cations, depending on their activity and the pH of the apoplast solution. There will always be free Ca^{2+} in equilibrium with this exchangeable Ca^{2+} in the matrix Since there is a very large free energy gradient directed towards the cell interior, an increase in the rate of Ca^{2+} entry via channels in the PM will draw in the free Ca^{2+} from the apoplast solution but this will be replaced rapidly by the Ca^{2+} buffer in the extracellular matrix. Thus, changes in the Ca^{2+} fluxes across the PM might be expected not to cause major shifts in the apoplasmic free Ca^{2+}.

In general, the results by Felle and Hanstein (this volume, pp. 295–306) and Plieth et al. (this volume, pp. 373–392) support this notion. There is, however, a very large internal stockage of vacuolar Ca^{2+}; this would seem to be the more obvious store for cytoplasmic homeostasis. The apoplast Ca^{2+} might be viewed as a buffer for Ca^{2+} at the outer surface of the PM and would, therefore, play an important role in maintaining membrane integrity.

5. THE APOPLAST AS THE FIRST LINE OF DEFENCE IN STRESS RESPONSES

Stresses in leaves can have a biotic or an abiotic origin. A leaf exists in air and in water that is rich in the spores of fungi and bacteria. Some of these are pathogenic and may have specialised means of invading the leaf by penetrating the epidermal layers. These are beyond the scope of the present work. The pores of open stomata are sufficiently large, however, to permit entry of airborne microbial propagules into the leaf. Systematic inspection of a number of plant species leads to the conclusion that microbes are of common occurrence in leaf tissues (Hallmann et al., 1977). Autotrophic nitrifiers are present in significant numbers in the needles of spruce trees (Teubner et al., this volume, pp. 405–426). Bacterial endophytes are found in intercellular spaces because they are too large to enter the pores of the wall

matrix. The apoplast solution provides C and N sources for bacterial growth, but the endophytic bacterial numbers are clearly kept in check either by substrate supply of by other means. In exchange for this substrate supply the plant may gain certain advantages. Some species of endophytic bacteria are dinitrogen fixers; much has been made of this but the consensus seems to be that N fixed in this way does not contribute more than 5% of the N requirements of species such as sugar cane (Dong et al., 1994).

Of more general significance is the suggestion that commensural microbes may increase resistance of the host to biotic stress (Hallmann et al., 1997), thus being analogous to a simple immune system. Plants can activate differentially distinct defence pathways depending on the type of challenge presented to them (Van Loon, 1997). This is an intriguing and complex subject but, in summary it can be said that the increased resistance can be of two kinds. Traumatic injury to cells in one part of the plant may trigger a defence pathway in which the accumulation of salicylic acid leads to the induction of pathogenesis-related, PR, proteins; the pathway is known as the systemic acquired resistance, SAR (Van Loon, 1997). This expression leads to an enhancement of the hypersensitive reactions in parts of the plant remote from the primary injury; these contain subsequent pathogenic attacks and limit their systemic development. A second kind of induced systemic resistance, ISR, has been extensively studied by van Loon and colleagues (Van Loon, 1997; van Wees et al., 2000). The non-pathogenic challenge presented by endophytic microbes, such as the rhizosphere bacterium. *Psuedomonas fluorescens*, activates an ISR pathway. In this, increased jasmonate level is a crucial signal eliciting the expression of PR genes and the eventual release of PR proteins. Most published work has been concerned with the exposure of roots to the bacteria, but ISR also occurs when they are applied to leaves (van Loon, personal communication).

Leaves can be subjected to intense biochemical oxidations and reductions during metabolism as well as experiencing atmospheric pollutants, such as ozone. These processes can lead to oxidative stress that needs to be dealt with if serious damage to cells is to be avoided. This has become a major research activity in recent decades (Dietz, 1997). Although the context is specifically related to excessive Mn supply via xylem delivery, in the paper of Fecht-Christoffers et al., (this volume, pp. 307–322), the response in the apoplast to the presence of a strong oxidant is an example of a more generalized set of responses. Substances such as ascorbic acid and dehydroascorbic acid, NADH-oxidase activity and peroxidases all of which are involved in the transformations of Mn in the apoplast. There is also the release of a raft of PR proteins with glucanase, chitinase activities into the apoplast of cowpea leaves; similar observations have been made in sunflower (Jung et al., 1995). The “defence” proteins are released in response to cell trauma (see above). The appearance of PR proteins in the

apoplast fluid suggests that high Mn levels may disrupt the integrity of cell membranes. Earlier work by Wissemeier and Horst (1987) showed that callose is deposited on walls of cowpea (*Vigna unguiculata*) subjected to excessive Mn. Callose is often an indication of disruption of PM integrity and of the normal controls over cellular Ca^{2+} homeostasis.

Many sorts of cell injury or stress lead to H_2O_2 production and events downstream of this seem, at present, to be of a rather general kind. The history of research into biochemical and physiological "cascades" shows that initially, a puzzling variety of responses follow from a single event. It is then questioned as to how a simple signal can generate such a diversity of biochemical and physiological consequences.

A classic example would be the consequences of elevated cytosolic calcium ion activities. Many different kinds of perturbation raise cell Ca^{2+} activity, some of which are described by Plieth et al. (this volume, pp. 373–392). Refinement of techniques revealed the subtleties of this elevation (Trewavas and Mahlo, 1998). We now know, for instance, that both the amplitude and frequency of transient elevations of cell calcium are important in what happens subsequently. A large, sustained spike of cell Ca^{2+} has a different effect to frequent smaller spikes. It seems likely that the cell is able to interpret the nature of the signal from information of this kind (Knight, 2000). Other responses to environmental signals and challenges have yet to be investigated as intensively as those that elevate cell calcium. When this has been done, it is highly probable that the responses in the apoplast will be seen to be more subtle and diverse than they appear at present.

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Section 1

Cell Wall–Ion Interactions: Significance for Nutrition of Plants and their Stress Tolerance

CELL WALL–ION INTERACTIONS

Significance for the nutrition of plants and their stress tolerance

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Key words: aluminium, boron, cell wall, ion interactions, pectin matrix, silica

In addition to its central role in plant growth and development, the cell wall is a plant's interface with the environment. This dynamic composite of cross-linking glycans embedded in a gel of pectin provides plant cells with a rich variety of shapes and sizes (McCann and Roberts, 1991). Different types of walls are made by flowering plants, a "Type I" pectin-rich, xyloglucan-cellulosic wall is made by most dicotyledonous and non-gramineous monocotyledonous plants, and a "Type II" pectin-poor, arabinoxylan-cellulosic wall is made by commelinoid monocots, including grasses and cereals (Carpita and Gibeaut, 1993). Unique to the walls of Poales species (grasses and cereals), is a mixed-linkage (1→3),(1→4)- β-D-glucan that appears transiently during cell expansion phases of growth (Carpita, 1996; Smith and Harris, 1999). Type I walls are further distinguished from Type II walls in the way their structures are reinforced when differentiation begins. The Type I is characterized by the appearance of several cross-linking structural proteins, such as the extensins and other kinds of hydroxyproline-rich glycoproteins, glycine-rich proteins, and proline-rich proteins (Cassab and Varner, 1988). Except in rare instances of tough cells, such as those of the maize periderm (Hood et al., 1988), the Type II wall has very little structural protein. Instead, the walls are cross-linked mostly by phenylpropanoids, such as esterified and etherified hydroxycinnamic acids and other aromatic lignin-like substances (Scalbert et al., 1985). This property imparts a strong autofluorescence in the non-lignified cells of the Type II, permitting a facile means to classify them (Rudall and Caddick, 1994).

B. Sattelmacher† and W.J. Horst (eds.), The Apoplast of Higher Plants: Compartment of Storage, Transport and Reactions, 15–18.

The pectin-rich Type I wall provides a complex, dynamic matrix that is the major determinant of wall pH, ionic balance, porosity, and electrical status (Carpita and Gibeaut, 1993). Pectins comprise two principal uronic acid-rich polymers, homogalacturonan (HG) and rhamnogalacturonan I (RG I). The HGs have a repeating backbone of α-D-(1→4)-linked galacturonosyl residues and are secreted to the wall in largely esterified form, with 70% or more of the residues bearing methyl esters. They may be de-esterified in muro, in random and block-wise fashion, at precise stages of cell development (Willats et al., 2001). Calcium ions play a key structural role once the methyl esters are removed. If long runs of unesterified uronosyl residues exist, then anti-parallel chains of HGs in are tightly cross-linked into "junction zones". Calcium can also cross-link parallel chains of partly de-esterified HGs (Carpita and Gibeaut, 1993).

HGs may also be substituted with neutral sugars, such as xylose to form xylogalacturonan enriched in flowering and fruit tissues, and/or acetylated (Schols et al., 1995). The HGs also form the backbone of one of the most complex polysaccharides found in Nature, called rhamnogalacturonan II (RG II). RG IIs contain many rare sugars and saccharinic acids, including apiose, aceric acid, Kdo and Dha, in four distinct side chains (O'Neill et al., 1990). One of the two apiose residues forms a boron-di-diester, coupling to RG II molecules, and forming structures essential for growth and development (O'Neill et al., 2001), porosity (Fleischer et al., 1999), and wall elasticity (Findeklee and Goldbach, 1996) and tensile strength (Ryden et al., 2003). The RG I is a polymer of the repeating disaccharide, →2)- α-L-rhamnosyl-(1→4)- α-D-galactosyl-(1→, which possesses neutral side-chains of highly branched (1→5)- α-L-arabinans, (1→4)- β-D-galactans, and type I arabino-(1→4)- β-D-galactans (Willats et al., 2001).

Given the enormous complexities and dynamics of the negatively charge pectin matrix, it is not surprising that it plays a central role in the mineral nutrition and ion status of plants, both at the sites of absorption in the root and utilization at the shoot meristems. This section contains four articles that describe unique features of mineral nutrition and the impacts of environmental stress. Two ions, boron and silica, are considered to impact structural elements of the wall directly. Wimmer and Goldbach (this volume, pp. 19-32) review boron in the type I cell wall and provide new data on its interactions with calcium ions in the formation of boron complexes. Because the only known function for boron in the apoplast is in the dimerization of RG II molecules (Ishii and Mansunaga, 1996; O'Neill et al., 1996), and given the function of these dimers in wall architecture (Findeklee and Goldbach, 1996; Fleischer et al., 1999; Ryden et al., 2003), divalent cations and other ions have important functions in regulating monomer to dimer ratios. In fact, Ca^{2+} has been shown to stabilize boron-RG II dimers (Kobayashi et al.,

1999). Wimmer and Goldbach (this volume, pp. 19–32) document such an interaction with free calcium. Within minutes of boron deficiency, stabilizing boron and calcium complexes form, resulting in retention of the remaining boron. Sodium ions interfere with these complexes, indicating that a consequence of saline stress could be the loss of boron from the apoplast. In addition to their studies of calcium-boron interactions, the authors show that boronic acid coupled to fluorescent dyes are new tools to locate the boron binding sites in muro.

An often overlooked element is silicon, which is accumulated to considerable amounts in several species. As Wiese et al. (this volume, pp. 33–48) describe, silicon is not known as an essential element, but has many direct and indirect beneficial effects through growth and development. The near impossible task of generating silicon-free plants precludes determination of its necessity. Silicon is absorbed as $Si(OH)_4$ and accumulates mainly in the apoplast. Wiese et al. (this volume, pp. 33–48) explain that plants range from "excluders" to "accumulators", the latter of which include many grass species with Type II walls, with cucumber being an exception among Type I-walled plants in accumulating silicon. Their article focuses on three silicon-mineral interactions: the impact of silicon on phosphate-induced zinc deficiency, on exchange capacity and binding forms of manganese, and on interactions with boron.

The key enzyme in creating a charged pectin matrix that carries cationic exchange capacity is pectin methyl esterase (PME). Thus, controls of this activity in the apoplast are essential determinants of the whole of mineral nutrition. Because they are cationic at physiological pH, polyamines could interact directly with de-methylated uronosyl residues of pectic substances. Gerendás (this volume, pp. 67–84) provides an extensive review of PME activity and some of the downstream consequences with respect to charge density, metal ions, calcium ions, and matrix pH. He focuses on the possible role of polyamines in regulation of PME activity. Levels of polyamines in the apoplast are greatly influenced by nutritional status, but very little if any direct effect on PME activity could be demonstrated.

While several authors point out that too much of an essential element can be as deleterious as too little, one of the principal agronomic problems today is aluminium (Al^{3+}) toxicity in acidic soils. Horst et al. (this volume, pp. 49–66) examine further the mechanism of Al^{3+} toxicity in root systems. They point out that the actual mechanism of toxicity remains to be established, although the cell wall is thought to play a role. One of the principal effects of Al^{3+} toxicity is a rapid inhibition of root elongation with a switch from cellulose synthesis to callose synthesis. Horst et al. (this volume, pp. 49–66) show a tight quantitative correlation between the levels of Al^{3+} and callose synthesis. They also discuss other factors involved in tolerance and resistance.

The pectin matrix has been implicated in contributing to resistance, presumably by binding to the Al^{3+} ion (Blamey et al., 1990), and Horst et al. (this volume, pp. 49–66) correlate Al^{3+} and pectin content. However, a role for pectins in resistance is tempered by the fact that Al^{3+}-tolerant grass species possess a pectin-poor Type II wall. Roles for organic acids, such as citric acid, and Ca^{2+}, silicates and phosphorous in mitigating Al^{3+} toxicity through sequestration is discussed.

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BORON IN THE APOPLAST OF HIGHER PLANTS

Relevance for rapid deficiency reactions, interaction with calcium activity, and characterization of soluble boron complexes

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Abstract. The relevance of different apoplastic B fractions for rapid B deficiency reactions and their interaction with apoplastic Ca activity was investigated. Soluble apoplastic B was reduced quickly under B deficiency in both, roots and leaves, and could be used as a tool to detect early or latent B deficiency. The critical level of soluble B was determined to be below 0.5 μM in *Vicia faba* roots. Negatively charged B complexes are potential reaction sites for polyvalent cations. In *V. faba* root tips, short term and long term interactions between B and Ca were observed. Within minutes of B deficiency, an immediate and mutual stabilizing interaction occurred between B and free Ca. A reduction of membrane-bound Ca was seen after 4 h of B-deficient conditions. In B-sufficient roots, 5% of total B was present in the form of a Ca-stabilized soluble B complex, which disappeared within 2 h of B deficiency. Besides soluble B, this B complex is likely involved in early B deficiency reactions. Results indicate that this complex could represent cell-wall precursors. An interaction between Na and Ca is likely involved in a reduction of B binding in the root apoplast under saline conditions and could be one of several factors influencing B tolerance. Boronic acid-coupled fluorescent dyes are shown to be useful tools to visualize B binding sites *in vivo*, and a novel technique using immunogold-labelled antibodies might allow the sub-cellular localization of B binding sites in the future.

Key words: boron deficiency, toxicity, soluble boron, calcium, boron complex, FITC

B. Sattelmacher† and W.J. Horst (eds.), The Apoplast of Higher Plants: Compartment of Storage, Transport and Reactions, 19–32.

1. INTRODUCTION

The apoplast harbours 50–98% of plant B, depending on species, plant organ and B supply. Symptoms of B deficiency are related to an impaired structural integrity of the cell wall (Goldbach, 1997; Loomis and Durst, 1992). Only recently the essentiality of B-RG II cross-links for Arabidopsis growth was shown (O'Neill et al., 2001) and remains the only demonstrated function of B in plants. Boron cross-links have been shown to affect the cell-wall pore-size (Fleischer et al., 1998, 1999) as well as the cell-wall tensile-strength (Ryden et al., 2003). Other possible functional sites include cell membranes and the cell-wall cytoskeleton continuum (Bassil et al., 2004; Goldbach et al., 2001; Yu et al., 2001, 2002, 2003), which may be indirectly affected by altered cell-wall physics (Findeklee and Goldbach, 1996; Fleischer et al., 1999) or a hence unknown mechanism with other ligands than RGII (Ralston and Hunt, 2001).

It was the objective of this project to improve our understanding of primary functions of B in higher plants. We aimed at determining soluble B concentrations and their relevance for early B deficiency reactions, as well as investigating the interaction between B and Ca in the apoplast. Based on obtained results, we then focused on the characterization of Ca-stabilized B fractions and their relevance for B tolerance. In a third part B binding sites were visualized using fluorescent dyes and the suitability of the method assessed.

Experiments were conducted using *Vicia faba* L. cv. Troy plants grown at 10 µM (+B) or without B (–B). An enlarged apoplastic fluid was obtained from roots by equilibration with different solutions. In leaves, apoplastic washing fluid was collected using a modified infiltration/centrifugation technique (Mühling and Sattelmacher, 1995). A spectrophotometric curcumin method was miniaturized in order to allow determination of B in small sample volumes (50–150 µl) with high sensitivity (detection limit 0.003 mg B l^{-1}) (Wimmer and Goldbach, 1999b). Reproducibility is good with a relative standard deviation of 1–5% at B concentrations between 0.05 and 0.40 mg B l^{-1}. The method has successfully been applied to determine B concentrations in waters, nutrient solutions, plant parts, phloem sap, xylem sap and apoplastic washing fluids of several plant species. Results are comparable to those determined with ICP-MS.

2. EARLY BORON DEFICIENCY REACTIONS

2.1 Chemical form of boron in the apoplast

Critical for determining B functions in the apoplast is an understanding of the physical and chemical properties of B and its complexes (reviews in

Brown et al., 2002; Goldbach, 1997; Loomis and Durst, 1992). Boric acid and borate can readily form esters with a variety of compounds possessing vicinal cis-diols or proximal hydroxyls in the correct orientation. Reactions are spontaneous, pH-dependent and rapid. A range of sugars, polyols, phenolics, amino acids and several other biomolecules have been predicted or shown to react with boric acid, although the stability of B complexes with these compounds under physiological conditions is likely low (Loomis and Durst, 1992; Ralston and Hunt, 2000, 2001). The functional significance of these putative B-containing complexes has not yet been determined.

Within the last few years, the chemical form of cell wall-bound B has been identified. A B polysaccharide complex was isolated from different plants and identified as a dimeric B-Rhamnogalacturonan II (dB-RGII) complex, where two chains of monomeric RGII (mRGII) are cross-linked by a 1:2 borate ester with two of the four apiosyl redidues of RGII side chains (Ishii and Matsunaga, 1996; Kaneko et al., 1997; Kobayashi et al., 1996; Matoh et al., 1993; O'Neill et al., 1996; Pellerin et al., 1996). Although mRGII is present in cells adapted to low B levels (Pellerin et al., 1996), B seems to be associated solely with the dimeric form (Ishii et al., 1999). Even though B-RGII accounts for 40–80 % of apoplastic B, the remaining 20–60 % have neither been identified, nor do we know their functions.

2.2 Relevance of apoplastic B concentrations for early deficiency reactions

Because of the rapidity and wide variety of B deficiency symptoms, it is a great challenge to determine primary functions of B in plants (Blevins and Lukaszewski, 1998). In growing tissues, primary effects of B deficiency occur within few minutes, but separating primary from secondary reactions remains difficult (Findeklee and Goldbach, 1996; Goldbach et al., 2001).

In *V. faba* roots, total B of older root parts decreased more slowly under B deficiency than that of growing root tips (Table 1), confirming the high stability of mature B-RGII complexes reported earlier (O'Neill et al., 1996). We therefore suggest that changes in soluble apoplastic B rather than changes in B-RGII complexes are responsible for early deficiency reactions and that a "critical level" of soluble B needs to be maintained at all times.

Table 1. Total B content (µg B/g DW) in V. faba, means of 6 replicates ± S.D.

	+B	-B 10 h	-B 24 h
Shoots	15.4 (±1.4)	n.d.	15.5 (±1.9)
Roots, older parts	17.5 (±3.4)	19.8 (±6.7)	13.5 (±3.2)
Roots, tips	14.3 (±3.3)	11.6 (±4.7)	4.6 (±2.8)*

* $p<0.001$